

IN THE CLAIMS

1. (original) A method for synthesizing a target polynucleotide that is efficiently expressed in a host-vector expression system, comprising the steps of:

(1) conducting a first polymerase chain reaction on a first template with a first primer pair to obtain a first polymerase chain reaction product; which is characterized in that the first template is any template sequence commonly used in the host-vector expression system or a fragment of the target polynucleotide;

(2) conducting multi-cyclic polymerase chain reactions by a primer extension technique to obtain a product comprising the target polynucleotide sequence; wherein the template used in each polymerase chain reaction is the product obtained in the previous polymerase chain reaction; and

which is characterized in that the primer pairs used in the polymerase chain reactions are designed to be any one of the following three primer pairs:

(i) the forward primer having two parts:

(a) the part (a1), locating at the 5' end region of the forward primer, comprising a fragment having more than 10 nucleotides and being homologous to the fragment at the 3' end region of the target polynucleotide sequence, and

(b) the part (b1), locating at the 3' end region of the forward primer, comprising a fragment having more than 10 nucleotides and being homologous to the sequence of the more than 10 nucleotides from the 5' end region of the template sequence;

and wherein the 3' end of the part (a1) is adjacent to the 5' end of the part (b1); and

the reversed primer having, at the 3 end region of the reversed primer, a fragment having more than 5 nucleotides and being capable of annealing to the 3 end region of the template sequence;

(ii) the forward primer having at the 3 end region of the forward primer, a fragment having more than 5 nucleotides and being homologous to the 5 end region of the template sequence; and

the reversed primer having

(a) the part (a2), locating at the 5 end region of the reversed primer, comprising a fragment having more than 10 nucleotides and being complement to the 5 end region sequence of the target polynucleotide sequence;

(b) the part (b2), locating at the 3 end region of the reversed primer, comprising a fragment having more than 10 nucleotides and be capable of annealing to the sequence of the more than 10 nucleotides from the 3 end region of the template sequence,

and wherein the 3 end of the part (a2) is adjacent to the 5 end of the part (b2); and

(iii) the forward primer having

(a) the part (a3), locating at the 5 end region of the forward primer, comprising a fragment having more than 10 nucleotides and being homologous to the fragment at the 3 end region of the target polynucleotide sequence;

(b) the part (b3), locating at the 3 end region of the forward primer, comprising a fragment having more than 10 nucleotides and being homologous to the

sequence of the more than 10 nucleotides from the 5 end region of the template sequence and wherein the 3 end of the part (a3) is adjacent to the 5 end of the part (b3); and the reversed primer having

(c) the part (c3), locating at the 5 end region of the reversed primer, comprising a fragment having more than 10 nucleotides and being complement to the 5 end region of the target polynucleotide sequence;

(d) the part (d3), locating at the 3 end region of the reversed primer, comprising a fragment having more than 10 nucleotides and annealing to the sequence of the more than 10 nucleotides from the 3 end region of the template sequence; and wherein the 3 end of the part (c3) is adjacent to the 5 end of the part (d3); and wherein all of the fragments of the target polynucleotide sequence used in the polymerase chain reactions in sequence constitute the target polynucleotide sequence; and

(3) obtaining the polynucleotide product comprising the target polynucleotide sequence from the final product of the multi-cyclic polymerase chain reactions.

2. (original) The method according to Claim 1, further comprising a step of removing the nucleotide sequence of the first template from the final product in the step (3) so as to obtain the target polynucleotide sequence if the first template is irrelevant to the target polynucleotide sequence.

3. (original) The method according to Claim 2, wherein the first template is designed to have restriction enzyme recognition sites at the both ends.

4. (original) The method according to Claim 1, wherein the fragment having more than 10 nucleotides used in each step is more than 15 nucleotides.

5. (original) The method according to Claim 1, wherein if the target polynucleotide sequence is heterogeneous to the host used in expressing the protein encoding the target polynucleotide, some codons of the target polynucleotide are changed to the codons which have a high expression efficiency in translating the same amino acid in the host cell.

6. (original) The method according to Claim 1, wherein the host is an enteric bacterium.

7. (currently amended) A method for highly expressing a target heterogeneous polypeptide encoded by a target polynucleotide in a host, which comprises the steps of:

(1) providing a target polynucleotide obtained by the method according to ~~any one of Claims~~ Claim 1 to 5;

(2) transforming or transfecting the target polynucleotide to the host; and

(3) expressing the target heterogeneous protein in the transformed or transfected host.

8. (original) The method according to Claim 7, wherein the host is an enteric bacterium.

9. (original) The method according to Claim 8, which further comprises, in the fragments of the target polynucleotide used for expressing the target heterogeneous polypeptide, changing the codon CTA encoding leucine to CTG, CTT, CTC, TTG, or TTA; the codon ATA encoding isoleucine to ATC or ATT; the codons CGG, AGG, AGA encoding arginine to CGT or CGC; the codon GGA encoding glycine changed to GGT or GGC; the codon CCC encoding proline to CCG, CCA or CCT; the codon CTA encoding leucine to CTG, CTT, CTC, TTG, or TTA; the codon ATA encoding isoleucine to ATC or ATT; the codons CGG, AGG, AGA encoding arginine to CGT or CGC; the codon GGA encoding glycine to GGT or GGC; or the codon CCC encoding proline to CCG, CCA or CCT.

10. (original) The method according to Claim 1, wherein the target polynucleotide encodes a mutated protein which has multiple mutation sites comparing to the wild-type form thereof.

11. (original) The method according to Claim 1, wherein the first polymerase chain reaction in the step (1) further conducted by a helper primer which is homologous to one primer of the first primer pair and identical to a fragment of one strand of the target polynucleotide.

Please add the following new claims:

12. (new) A method for highly expressing a target heterogeneous polypeptide encoded by

a target polynucleotide in a host, which comprises the steps of:

- (1) providing a target polynucleotide obtained by the method according to Claim 2;
- (2) transforming or transfecting the target polynucleotide to the host; and
- (3) expressing the target heterogeneous protein in the transformed or transfected host.

13. (new) A method for highly expressing a target heterogeneous polypeptide encoded by a target polynucleotide in a host, which comprises the steps of:

- (1) providing a target polynucleotide obtained by the method according to Claim 3;
- (2) transforming or transfecting the target polynucleotide to the host; and
- (3) expressing the target heterogeneous protein in the transformed or transfected host.

14. (new) A method for highly expressing a target heterogeneous polypeptide encoded by a target polynucleotide in a host, which comprises the steps of:

- (1) providing a target polynucleotide obtained by the method according to Claim 4;
- (2) transforming or transfecting the target polynucleotide to the host; and
- (3) expressing the target heterogeneous protein in the transformed or transfected host.

15. (new) A method for highly expressing a target heterogeneous polypeptide encoded by a target polynucleotide in a host, which comprises the steps of:

(1) providing a target polynucleotide obtained by the method according to Claim 5;

(2) transforming or transfecting the target polynucleotide to the host; and

(3) expressing the target heterogeneous protein in the transformed or transfected host.